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Studies on the Ameliorating Effects of Oxygenated Fatty Acids on Lipid Metabolism(Digest_要約)

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GENERAL INTRODUCTION

Nowadays, dysfunction or imbalance of lipid metabolism is believed as the cause of many lifestyle related diseases, such as obesity, fatty liver and atherosclerosis. These diseases consequently are the main risk factors of cardiovascular disease (CVD) which is the leading cause of mortality worldwide. The prevalence of CVD has not increased only in the elder, but also in the midlife and the young adulthood due to the changes of the lifestyle in our societies. Hence, the improvement of lipid metabolism is essential to prevent or ameliorate many diseases. One of the most important factors that are concerned as the therapeutic target of CVD is triacylglycerol (TG).

TG is a dense form in which to store the energy. It circulates in the plasma in lipoproteins and also presents as lipid droplets within cells. Most tissues are involved in TG metabolism, but adipose tissue, skeletal muscle and liver are quantitatively more important than other tissues. In the case of adipose tissue, adipocyte hypertrophy results in elevated TG storage, primarily contributes to the development of obesity. Whereas in the liver fatty acids were re-esterified within the endoplasmic reticulum (ER) to produce TG that will be secreted as very-low-density lipoprotein (VLDL). Therefore, ameliorating the dysfunctions of lipid metabolism in the adipose tissue and the liver is essential to abate diverse lifestyle related diseases, particularly CVD.

Generally, diet from marine source shows many benefits for health. Omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from marine sources, have been developed commercially as dietary supplements due to their various health benefits, particularly their hypolipidemic and anti-obesity effects. EPA has TG-reducing effects in normolipidemic and in hyperlipidemic subjects. However, EPA in the diet is easily oxidized at room temperature and several types of oxidized EPA (OEPA) derivatives are generated. It has

been proposed that oxidation products of omega-3 fatty acids, especially EPA and DHA, play crucial roles in the palliative effects via many mechanisms particularly those that are cardiovascular-related.

On the other hand, some PUFAs, such as linoleic acid (LA) and linolenic acid (LNA), cannot suppress hepatic lipogenesis and TG accumulation in mouse adipocytes (3T3-L1). Notwithstanding, it is suspicious that their oxygenated forms might involve in many benefits in our health. Recently, Ogawa et al. discovered many species of intermediates from the metabolic pathway of gut bacterium *Lactobacillus plantarum*, which are hydroxy-fatty acids, oxo-fatty acids, conjugated fatty acids, and partially fatty acids. These new-finding fatty acids were predicted to exert their physiological functions, especially the improvement in lipid metabolism.

The aims of this study were to examine the properties and bioactivities of oxygenated fatty acids from various sources, including the autooxidation of EPA and the fermentation from *L. plantarum*, on lipid metabolism in human hepatocellular liver carcinoma cell line (HepG2), 3T3-L1 preadipocytes and diabetic KK-Ay mice fed with high fat diet. In this study, for the first time the ameliorating effects of OEPA and oxygenated fatty acids from *L. plantarum* fermentation on TG accumulation were investigated. The importances of this study are not only the preferable effects on lipid metabolism of oxygenated fatty acids from marine sources compared to their intact form, but the data also suggested that the efficiency of each oxygenated fatty acid depends on the chemical structure, particularly the type, the location and the number of functional group and double bond.

Chapter 1: Oxidized EPAs (OEPAs) more potently suppress lipogenesis in hepatocytes than its intact form

INTRODUCTION

CVD is the leading cause of mortality worldwide. TG, in either the serum or the liver, is a major risk factor for CVD. With respect to the importance of hepatic TG levels, nonalcoholic fatty liver disease (NAFLD) is highly associated with CVD. Several prospective epidemiological studies recently demonstrated that both an increased liver enzyme concentration in the serum and hepatic steatosis determined by ultrasound are able to predict the development of CVD independent of alcohol consumption or traditional CVD risk markers, such as serum LDL cholesterol concentrations. In addition, Rijzewijk et al. demonstrated that type 2 diabetes mellitus patients with high liver TG content showed a decreased myocardial perfusion compared with similar diabetes patients with low liver TG content. Therefore, the dysfunction of hepatic lipid metabolism has been of concern as a therapeutic target of CVD.

Omega-3 PUFAs, especially EPA, have been developed commercially as dietary supplements due to their various health benefits, particularly their ameliorating effect on CVD [11, 12]. EPA has TG-reducing effects in normolipidemic and in hyperlipidemic subjects. It has been proposed that EPA decreases TG through the regulation of peroxisome proliferator-activated receptor α (PPAR α) and sterol regulatory element-binding protein (SREBP)-1, which govern hepatic fatty acid (FA) catabolism and synthesis, respectively.

Oxidation products of omega-3 fatty acids, especially EPA and DHA, play crucial roles in the palliative effects via many mechanisms especially those that are cardiovascular-related. A study by Majkova et al. showed that components of oxidized docosahexaenoic acid (DHA) can alleviate the endothelial dysfunction caused by coplanar PCB77. Furthermore, oxidized EPA (OEPA) has been shown to inhibit leukocyte-endothelial interactions by potently activating PPAR α in endothelial cells, and

to do so to a much greater extent than native EPA. Although native EPA activates PPAR α about half as well as OEPA, unlike EPA, OEPA has effects on leukocyte-endothelial interactions *in vitro* and *in vivo*. 5-HEPE, a metabolite produced from EPA in human neutrophils and eosinophils, has been shown to be a potent agonist for G protein-coupled receptor (GPR) 119, which results in a reduction in food intake and in body weight gain in rats, and to enhance glucose-dependent insulin secretion.

However, the effects of OEPA on many processes are still obscure. Therefore, the aim of this study is to elucidate the mechanism of 4-24 h OEPAs which are composed of various species of oxidation products on lipid metabolism, particularly via the LXR α and SREBP-1c pathway, which plays an important role in lipid metabolism in liver cells. Surprisingly, the result shows that OEPA significantly down-regulates the expression of lipogenic genes, which results in the suppression of hepatocellular TG more than EPA.

MATERIALS AND METHODS

OEPAs, prepared by incubation in a 40°C water bath for 4-24 h, were analyzed to characterize their derivatives by LC-MS and then treated to human hepatocellular liver carcinoma cell line (HepG2) with T0901317, synthetic LXR agonist. Cell viability was determined by WST-1 assay. The mRNA expression levels of sterol-regulatory element binding protein-1c (SREBP-1c), stearoyl-coenzyme A desaturase-1 (SCD-1), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC1), glycerol-3-phosphate acyltransferase (GPA) and peroxisome proliferator-activated receptor γ co-activator 1 β (PGC-1 β) were evaluated by real-time RT-PCR. The precursor and mature forms of SREBP-1 protein were examined by western blot analysis. Cellular TG accumulation was assayed by using Folch's technique and TG E-test kit.

RESULTS

I. Changes in derivatives of EPA oxidation products after autoxidation

First, OEPA_s were prepared from EPA (10 mg) by autoxidation. [M-H]⁻ at the mass to charge ratio (m/z) 301.2 presented as the molecular ion of EPA and m/z 317.2-397.2 were presumably derived from EPA oxidation products. The intensity of the molecular ion of EPA at m/z 301.2 decreased continuously after autoxidation. After oxidation for 4 h, several new ions that were oxidative products of EPA appeared. The molecular ion [M-H]⁻ of hydroxy-EPA (HEPE) at m/z 317.2 reached a peak at 4 h incubation and then gradually decreased. HEPE was the major EPA oxidation product at 4 and 8 h. On the other hand, the ion at m/z 349.2 was detected at 8 h and after that it reduced gradually. The ions at m/z 333.2, 365.2 and 397.2 were found at 4, 4 and 8 h, respectively, and then they tended to increase gradually (Fig. 1).

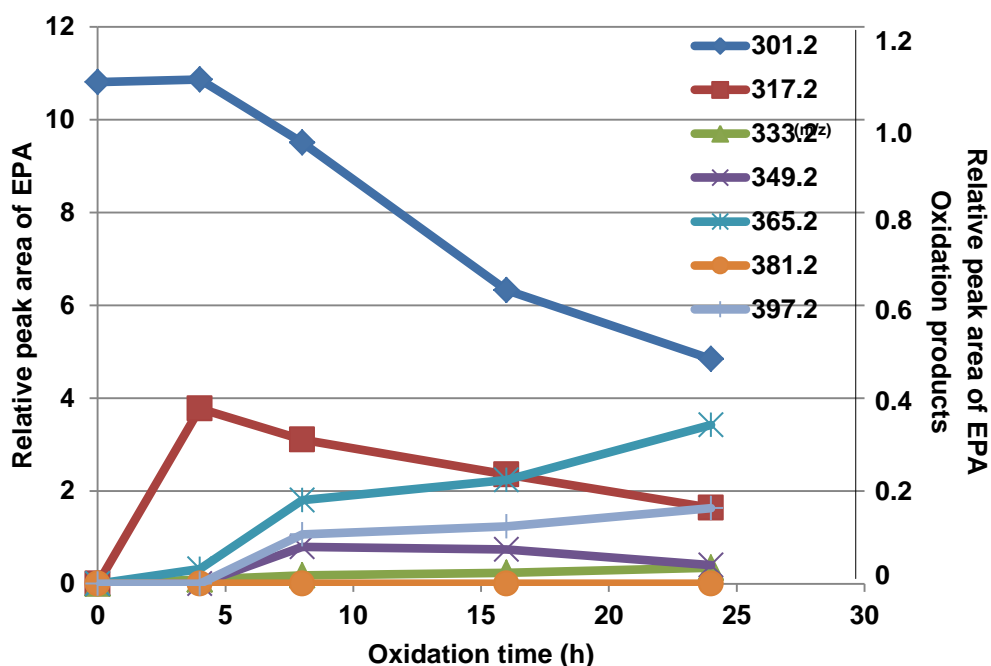


Figure 1. Ion intensities derived from EPA and EPA oxidation products analyzed by LC/MS. m/z 301.2 was derived from EPA and m/z 317.2-397.2 were presumably derived from hydroxy- and hydroperoxy-EPA_s. Each oxidized EPA was prepared from EPA (10 mg) incubated in 40°C water bath for 4, 8, 16 or 24 h.

II. Effects of each fatty acid on HepG2 cell viability

Various concentrations of fatty acids and T0901317 were added to HepG2 liver cells in culture. Figure 2 shows the results of the viability assay after 24 h of treatment. The cytotoxic effect of EPA weakened depending on the oxidation time. EPA, 4 h OEPA, 8 h OEPA and 16 h OEPA at 30 and 60 μ M significantly reduced cell viability to around 24%, 34%, 34% and 66% of vehicle-treated control cells, respectively. The 24 h OEPA at 30 μ M, but not at 60 μ M, had no cytotoxic effect on HepG2 cells under these experimental conditions. Notwithstanding, when HepG2 cells were co-incubated with 10 μ M vitamin E, the cytotoxicity of EPA and OEPA was eliminated.

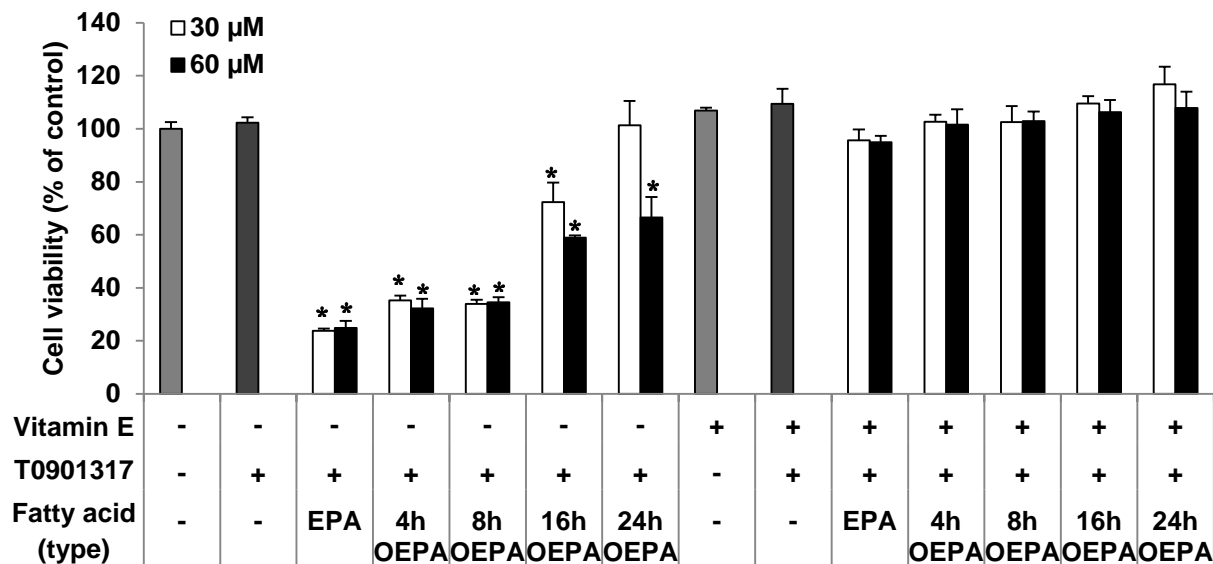


Figure 2. Effects of EPA and OEPA on cell viability as determined by the water soluble tetrazolium (WST)-1 assay. Each fatty acid (30 or 60 μ M) was added to HepG2 cells with T0901317 (10 nM) and/or vitamin E (10 μ M) in serum-free medium containing 0.1% BSA. The final ethanol concentration was 0.3%. After 24 h incubation, the WST-1 solution was added to the cells. The absorbance at 450 nm corresponds to cell viability. Expression levels are presented as relative percentage to vehicle control (ethanol). Data are reported as means \pm SD (n = 4/group). * A significant difference from the vehicle control group (p < 0.05).

III. OEPA suppresses TG synthesis in HepG2 cells

While the cells were being treated with each compound, vitamin E was simultaneously added in FBS-free medium containing 0.1% BSA since EPA has very severe cytotoxic effects, as shown in Figure 2. T0901317 significantly augmented the cellular levels of TG after 48 h of incubation. EPA significantly reduced TG synthesis of T0901317-treated HepG2 cells. Surprisingly, treatment with OEPA for 4, 8 and 16 h significantly inhibited the cellular TG content of HepG2 cells more than did EPA (Fig. 3). Moreover, the 24 h OEPA, which has around 50% the ion intensity at m/z 301.2 to intact EPA (Fig. 1), showed almost the same levels of TG suppression as did intact EPA (Fig. 3). These findings indicate that HEPEs might play a crucial role in the effect of EPA on the inhibition of cellular TG synthesis in HepG2 liver cells.

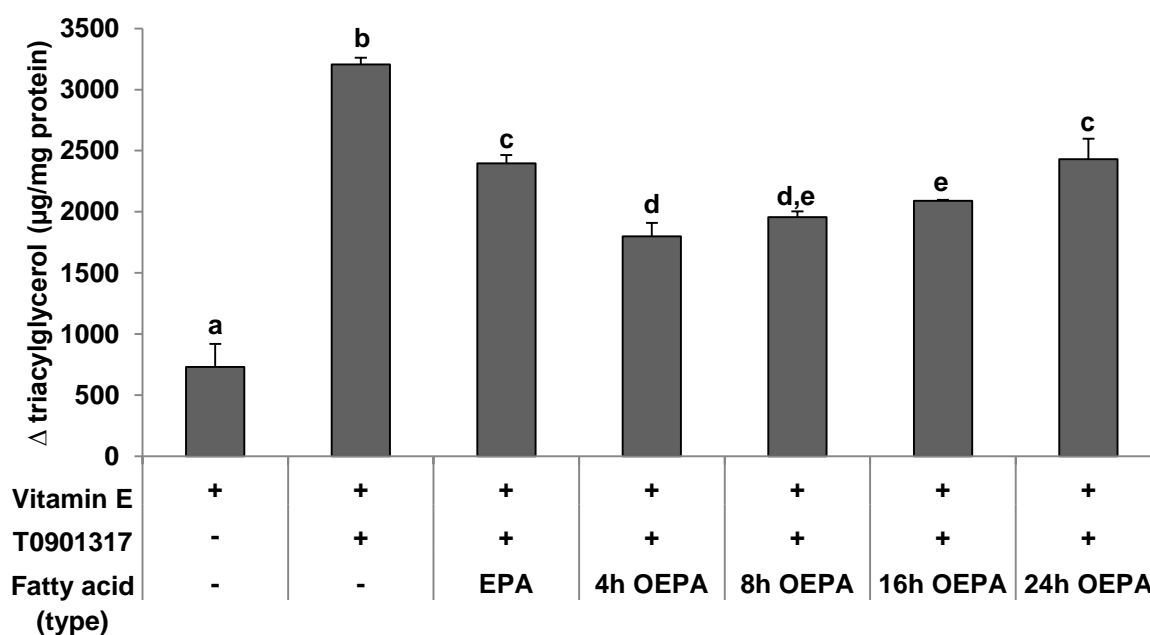


Figure 3. Effects of EPA and OEPA on TG synthesis in HepG2 cells. Each fatty acid (60 μ M) was added to HepG2 cells with T0901317 (10 nM) and vitamin E (10 μ M) as noted in serum-free medium containing 0.1% BSA for 48 h. The final ethanol concentration was 0.3%. The increased TG levels per mg protein (TG levels after 48 h of incubation minus the levels of reference cells) are shown. Data are reported as means \pm SD (n = 3-4/group). Values with different letters are significantly different (p < 0.05).

IV. OEPA decreases SREBP-1c mRNA expression and maturation more effectively than EPA

The mRNA expression levels of SREBP-1c and SREBP-1 protein levels were determined. The expression of SREBP-1c mRNA in cells treated with 10 nM T0901317, a synthetic LXR agonist, was 9.4-fold higher than in vehicle-treated control cells (Fig. 4). Treatment with EPA and 4-24 h OEPA significantly down-regulated SREBP-1c mRNA expression stimulated by T0901317. In agreement with the result of TG accumulation, the suppressive effect of OEPA at 4 and 8 h was statistically stronger than EPA. The 4 h OEPA treated cells showed the least SREBP-1c mRNA expression as also noted in the effect on TG synthesis in hepatocytes. Furthermore, the 24 h OEPA reduced SREBP-1c expression to the same level as treatment with EPA (similar to Figure 3).

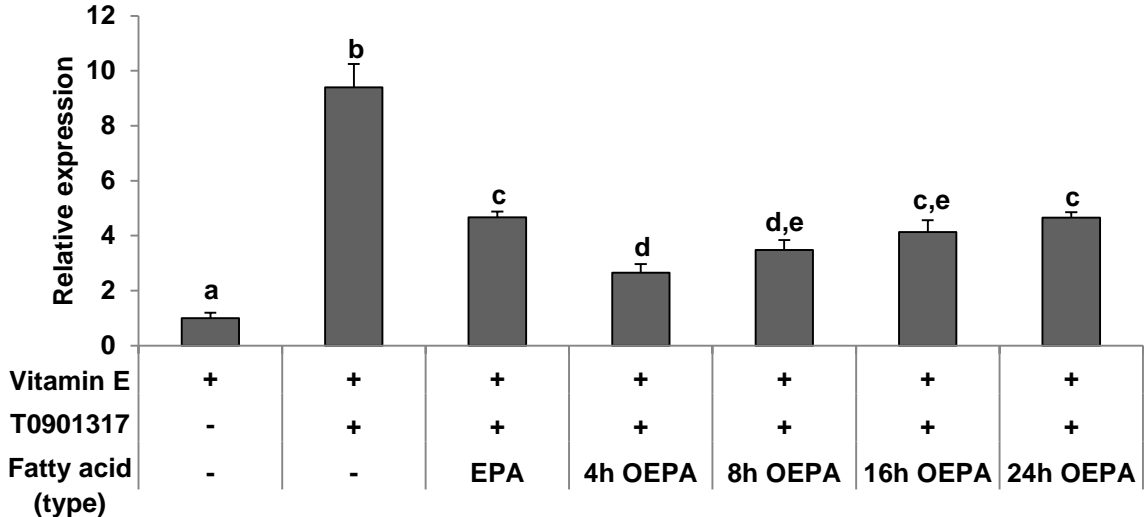
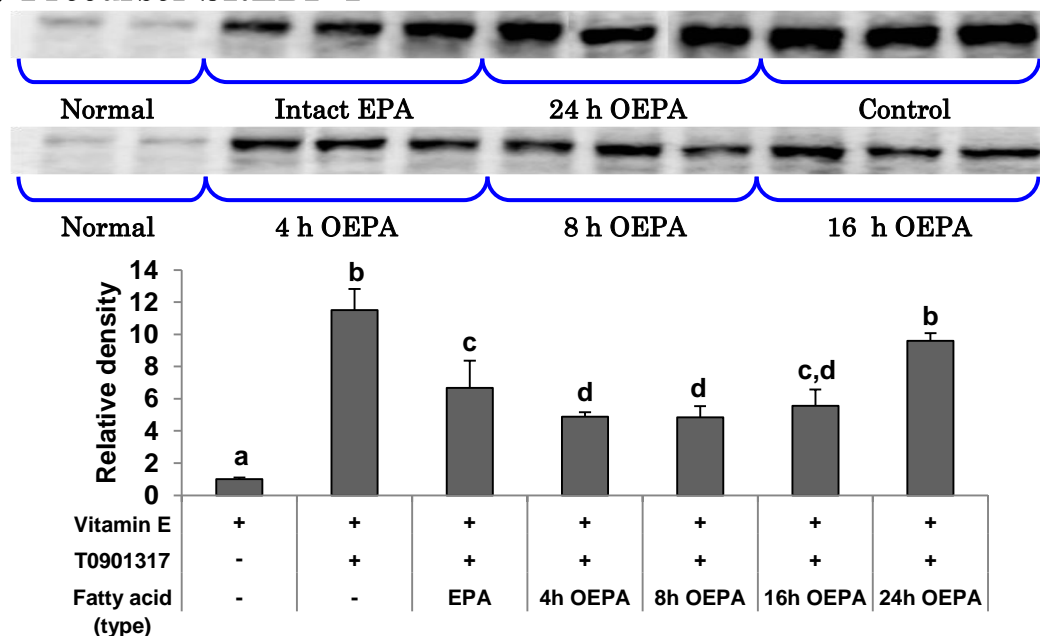


Figure 4. Effect on SREBP-1c mRNA expression. Each fatty acid (60 μ M) was added to HepG2 cells with T0901317 (10 nM) and vitamin E (10 μ M) as noted in serum-free medium containing 0.1% BSA. The final ethanol concentration was 0.3%. After 24 h incubation, SREBP-1c mRNA levels were quantified by real-time quantitative PCR. Each value of SREBP-1c mRNA was adjusted by that of 18s rRNA (internal control). Expression levels are presented as -fold induction relative to the vehicle control (vitamin E and ethanol). Data are reported as means \pm SD (n = 3/group). Values with different letters are significantly different (p < 0.05).

To investigate the effect of these fatty acids on SREBP-1 protein levels, the full-length precursor form in cell membranes (125 kDa) and the cleaved mature form (68 kDa) in nuclear extracts were estimated by immunoblotting. Because the antibody used cannot distinguish between the SREBP-1c and -1a isoforms, the general term SREBP-1 was used to refer to the results. Representative blots are shown in Figure 5A and B. T0901317 increased the levels of both the precursor and the mature forms of SREBP-1.

The 4-16 h OEPAs as well as EPA significantly decreased the T0901317 induction of both the precursor and mature forms of SREBP-1. On the other hand, the 24 h OEPA significantly differentiated only mature SREBP-1. Corresponding to SREBP-1c mRNA expression, the precursor form of SREBP-1 was down-regulated by treatment with the 4 and 8 h OEPA more significantly than EPA. Interestingly, the mature form of SREBP-1 was also inhibited by the 4 h OEPA more significantly than EPA and the vehicle control.

(A) Precursor SREBP-1



(B) Mature SREBP-1

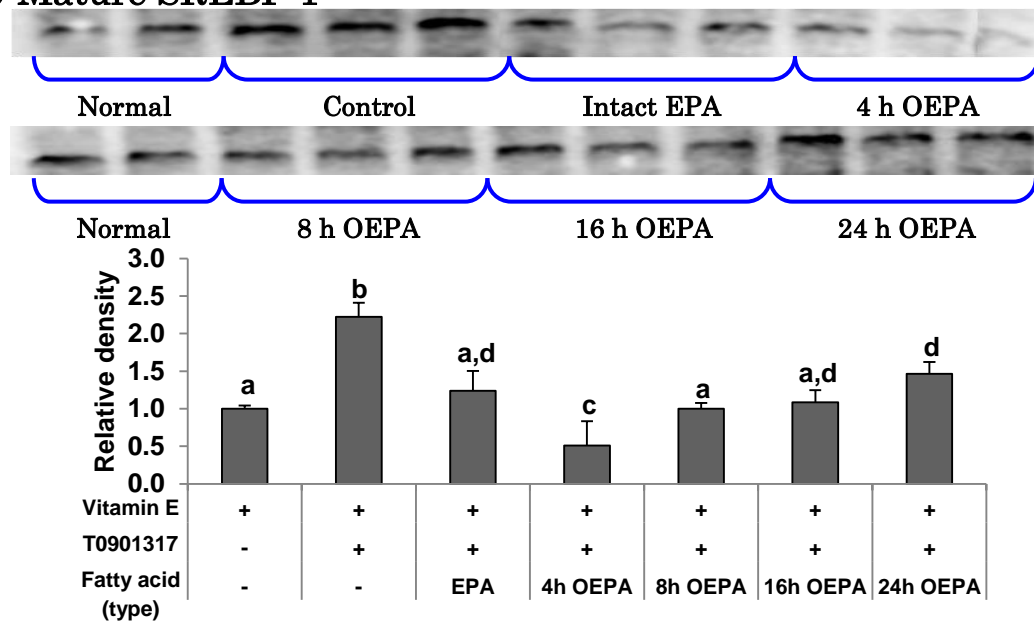


Figure 5. Effects on precursor (A) and mature (B) SREBP-1 protein expression. Each fatty acid was added to HepG2 cells with T0901317 (10 nM) and vitamin E (10 μ M) as noted in serum-free medium containing 0.1% BSA. The final ethanol concentration was 0.3%. The data represent the mean -fold change of the precursor and the mature forms of SREBP-1 from the vehicle control (ethanol). Data are reported as means \pm SD (n = 3-4/group). Values with different letters are significantly different (p < 0.05).

V. Regulation of mRNA levels of lipogenic genes by OEPA

Then SREBP-1c target genes and other lipid metabolism related genes were examined. Treatment with EPA or 4 h OEPA significantly decreased the expression of Acetyl CoA carboxylase (ACC), Fatty acid synthase (FAS) and Stearoyl-coenzyme A desaturase-1 (SCD1) mRNAs (Fig. 6A, B, C). Corresponding to the aforementioned results, treatment with 4 h OEPA significantly decreased ACC and FAS expression more than the vehicle control while treatment with EPA reduced the expression of these genes to the same level as the vehicle control (Fig. 6A, B). Moreover, 4 h OEPA treatment significantly down-regulated the T0901317-induced expression of SCD1 more than did EPA (Fig. 6C). GPA, an enzyme located in the endoplasmic reticulum and the

mitochondrial membrane that is required for TG synthesis in the glycerol phosphate pathway, which is regulated by SREBP-1c, was significantly weakened by 4 h OEPA compared to LXR α agonist-induced cells, while EPA did not affect the increase of GPA induced by T0901317 (Fig. 6D). In agreement with the decreased expression of lipogenic target genes of SREBP-1c, mRNA expression levels of ATP-binding cassette transporter A1 (ABCA1), the other target gene of LXR that is related to cholesterol transport, was significantly decreased by 4 h OEPA, but was not significantly changed by EPA (Fig. 6E). It was unexpectedly found that after the 4 h OEPA treatment, the expression of PGC-1 β , the co-activator of LXR α and the SREBP families, was significantly down-regulated in the T0901317-induced group. In contrast, EPA did not affect the expression of PGC-1 β (Fig. 6F).

VI. The members of HEPEs contained in EPA oxidation products after autoxidation

From the results mentioned above, the 4 and 8 h OEPAs, which contain high portions of HEPEs (m/z 317.2), showed dominant suppressive effects on the lipogenesis pathway in HepG2 hepatic cells induced by the synthetic LXR α agonist among EPA and other OEPAs which contain lower portions of HEPEs. For this reason, the standards of HEPEs, including 5-, 11- and 18-HEPEs, were utilized to identify the kinds of HEPEs that occurred in the autoxidized process by LC-MS. The results showed that 5-, 11- and 18-HEPEs might be members of HEPEs in OEPA due to their correlate retention time between peaks of the standards and HEPEs derivative in 4 h OEPA (Fig. 7A). Based on their peak area, 5-HEPE is the major member of HEPEs in 4 h OEPA (25.0%). Besides, 4 h OEPA also has 11-HEPE and 18-HEPE in the proportion of 14.1% and 12.3%, respectively, comparing with total peaks area of ion at m/z 318.4 (Fig. 7B).

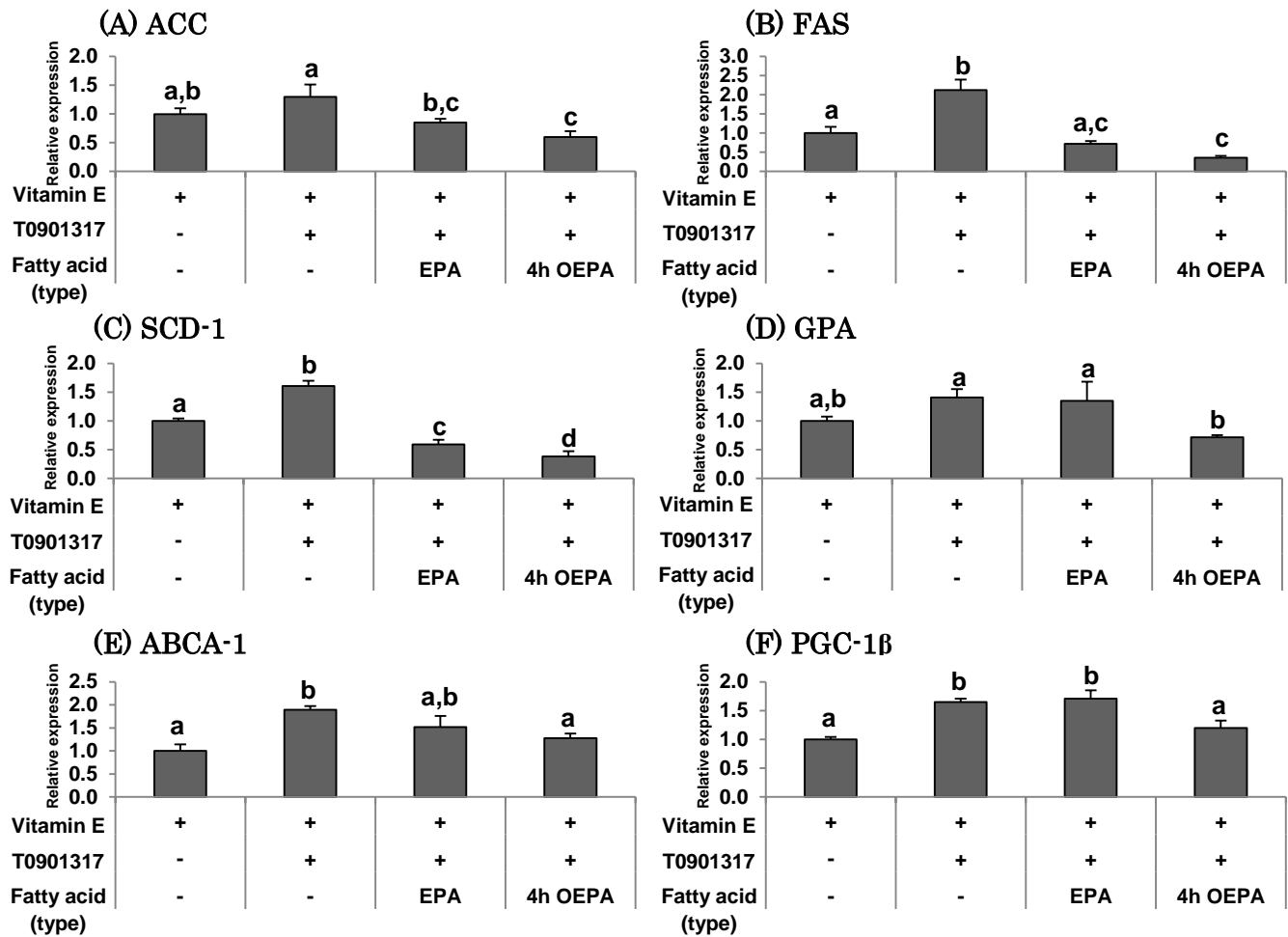
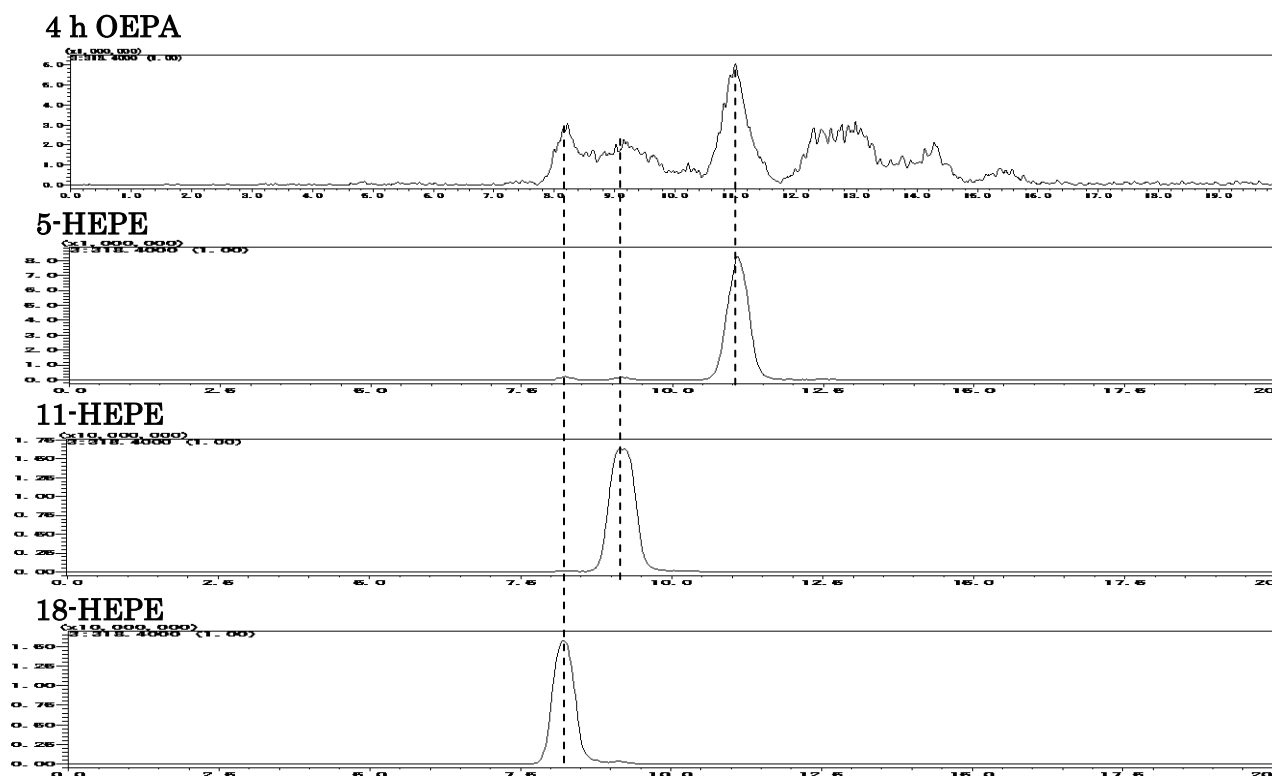


Figure 6. Effects of EPA and OEPA on the expression of acetyl-CoA carboxylase-1 (ACC; A), fatty acid synthase (FAS; B), stearoyl-coenzyme A desaturase-1 (SCD-1; C), glycerol-3-phosphate acyltransferase (GPA; D), ATP-binding cassette sub-family A member 1 (ABCA1; E) and peroxisome proliferator-activated receptor γ co-activator 1 β (PGC-1 β ; F) mRNAs. Each fatty acid (60 μ M) was added to HepG2 cells with T0901317 (10 nM) and vitamin E (10 μ M) as noted in serum-free medium containing 0.1% BSA. The final ethanol concentration was 0.3%. After 24 h incubation, ACC, FAS, SCD-1, GPA, ABCA1 and PGC-1 β mRNA levels were quantified by real-time quantitative PCR. Each value of ACC, FAS, SCD-1, GPA, ABCA1 and PGC-1 β mRNAs was adjusted by that of 18s rRNA (internal control). Expression levels are presented as -fold induction relative to the vehicle control (vitamin E and ethanol). Data are reported as means \pm SD (n = 3/group). Values with different letters are significantly different (p < 0.05).

(A) Selected ion chromatograms of m/z 318.4 (HEPEs) in 4 h OEPA compared with purified HEPEs



(B) Percentage of peaks area of each type of HEPEs in 318.4 m/z (hydroxyl EPA) of 4 h OEPA

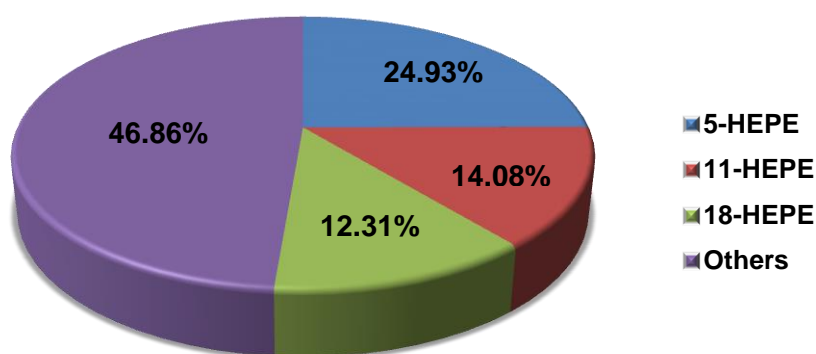
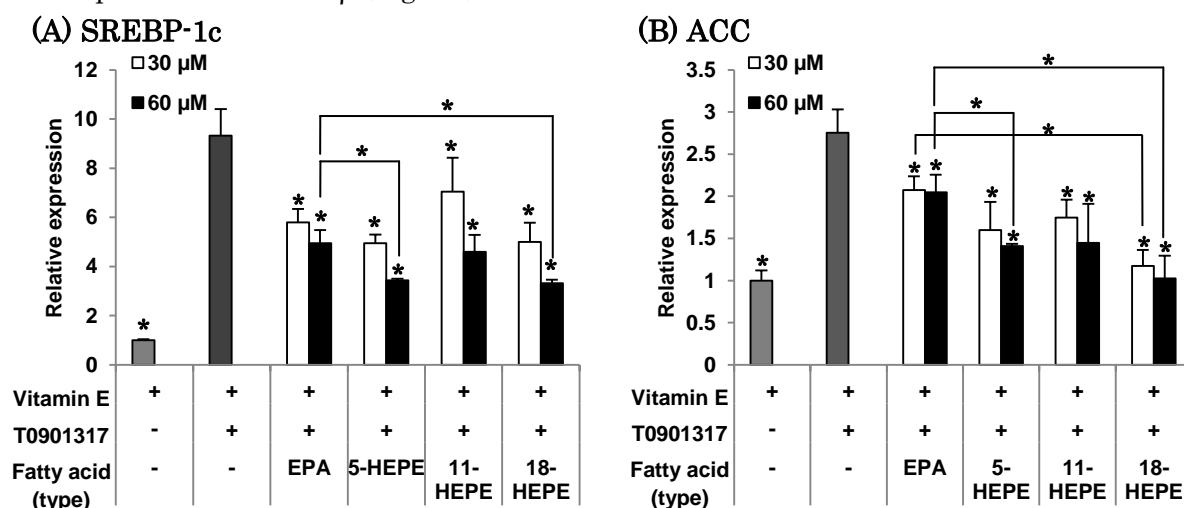


Figure 7. Various kinds of the standards of HEPEs were compared with HEPEs derived from autoxidized EPA. A: The retention time of 4 h OEPA, 5-HEPE, 11-HEPE, and 18-HEPE were allegorized. B: Ion intensities percentage of each type of HEPE contained in hydroxy EPA (m/z 317.2) derived from 4 h OEPA was measured by using the graph area from the specific retention time. Retention time and ion intensities in 4 h OEPA and HEPEs were analyzed by LC-MS.

VII. Regulation of lipogenic gene mRNA levels by 5-, 11- and 18-HEPEs

These standard HEPEs were then treated to HepG2 cells to confirm whether those HEPEs are involved in the TG reduction of OEPAs. At both 30 and 60 μM concentrations, all fatty acids significantly decreased the augmentation of SREBP-1c expression caused by the LXR α agonist. It was noteworthy that at a 60 μM concentration, 5-HEPE and 18-HEPE significantly reduced SREBP-1c expression more than did EPA (Fig. 8A). The expression of SREBP-1c target genes also had a tendency for the same responses. All fatty acids significantly down-regulated the T0901317-induced increase of ACC. At 30 μM , expression of ACC in the 18-HEPE treated group was significantly lower than in the EPA-treated group. In addition, 60 μM 5-HEPE or 18-HEPE significantly reduced the expression of ACC more than did EPA (Fig. 8B). Expression of SCD1 at both 30 μM and 60 μM 18-HEPE was significantly lower than was elicited by EPA at similar concentrations (Fig. 8D). On the other hand, all fatty acids significantly down-regulated the T0901317-induced expression of FAS, but there was no significant difference between any of the fatty acids at the same concentrations (Fig. 8C). Among all experimental groups, only treatment with 60 μM 18-HEPE significantly decreased GPA expression compared with T0901317-stimulated cells. Interestingly, cells which were treated with all types of HEPEs in this experiment had significantly reduced expression of GPA more than the EPA-treated cells (Fig. 8E). Similar to the 4 h OEPA treatment, 18-HEPE at a high dose can significantly decrease the expression of PGC-1 β (Fig. 8F).



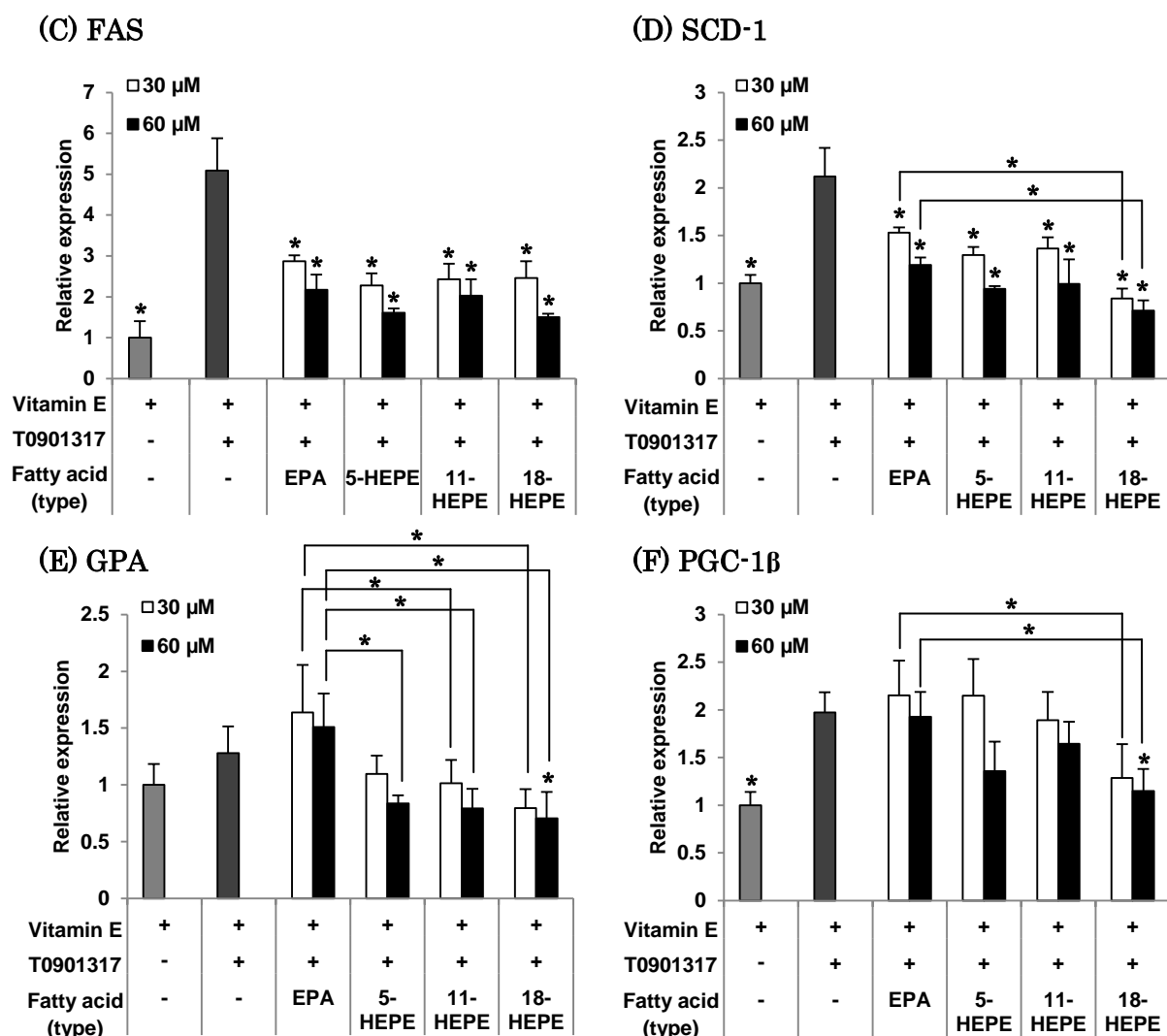


Figure 1-11. Effects of EPA, 5-HEPE, 11-HEPE and 18-HEPE on the expression of sterol-regulatory element binding protein-1c (SREBP-1c; A), acetyl-CoA carboxylase-1 (ACC; B), fatty acid synthase (FAS; C), stearoyl-coenzyme A desaturase-1 (SCD-1; D), glycerol-3-phosphate acyltransferase (GPA; E) and peroxisome proliferator-activated receptor γ coactivator 1 β (PGC-1 β ; F) mRNAs. Each fatty acid (60 μ M) was added to HepG2 cells with T0901317 (10 nM) and vitamin E (10 μ M) as noted in serum-free medium containing 0.1% BSA. The final ethanol concentration was 0.3%. After 24 h incubation, SREBP-1c, ACC, FAS, SCD-1, GPA and PGC-1 β mRNA levels were quantified by RT-PCR. The value of each mRNA was adjusted by that of 18s rRNA (internal control). Expression levels are presented as -fold induction relative to the vehicle control (vitamin E and ethanol). Data are reported as means \pm SD (n = 3/group).

* A significant difference from the T0901317-induced control group (p < 0.05).

DISCUSSION

Previous studies showed that oxidation products of EPA and DHA alleviate some pathways related to CVD. In this study, the hypolipidemic effect of OEPA on hepatic cells was emphasized. The hypolipidemic effect of PUFAs, including EPA, is attributable both to a decrease in lipogenesis and an increase in fatty acid catabolism through the regulation of SREBP-1 and PPAR α , respectively. Nevertheless, it is well known that PPAR α weakly expresses and has very low function in HepG2 cells, because the cells are originated from hepatoma and some kinds of genes, such as PPAR α , is mutated. Thus, this study focused on the regulation of lipogenesis via SREBP-1 by OEPA.

In summary, these data suggest that OEPA augments the ameliorating effect of EPA on lipogenesis in liver cells via the suppression of lipogenic genes related to SREBP-1c. In addition, the preferable ameliorating effect of OEPA on lipogenesis might be due to the decreases of the expressions of PGC-1 β and GPA by OEPA, meanwhile EPA does not significantly differentiate those expressions (Fig. 1-12). The findings provide insight into the importance of the location and the number of hydroxy groups of OEPA in the hypolipidemic effect. This study also suggests that oxidation products as food components might contribute to the beneficial effects of EPA on lipid metabolism in the liver resulting in the prevention of CVD. However, further *in vivo* experiments are necessary to ensure the effects of dietary OEPA on lipid metabolism.

Chapter 2: Oxygenated fatty acids fermented by *Lactobacillus plantarum* efficiently decrease TG synthesis in liver

Oxygenated fatty acids, extracted from intermediates of metabolic pathway of *L. plantarum*, were kindly provided from Dr. Prof. Ogawa (Laboratory of Fermentation Physiology and Applied Microbiology, Division of Applied Life Sciences, Graduate School

of Agriculture, Kyoto University). These oxygenated fatty acids were evaluated to HepG2 cells and diabetic KK-Ay mice fed with high fat diet (HFD). Cell viability was assessed by WST-1 assay. The mRNA expressions of SREBP-1c and other lipogenic genes were evaluated by real-time RT-PCR. The precursor and mature forms of SREBP-1 protein were fractionated and then examined by western blot analysis. Luciferase reporter assay was performed to study LXR α activity. Cellular TG accumulation was assayed by Folch's technique and TG E-test kit.

Diverse species of oxygenated fatty acids were screened and found that some specific species of them, which contains hydroxy or ketone group and some double bonds on the same location, prominently decreased SREBP-1c expression among all oxygenated fatty acids. Furthermore, these oxygenated fatty acids also statistically reduced both forms of SREBP-1c protein expression more than EPA and linoleic acid (LA) which is one of their precursors. They suppressed SREBP-1c through the regulation of LXR α as similar manner to EPA. These oxygenated fatty acids reduced the expression of SCD-1, FAS and ACC1 to almost the same levels with EPA, yet they significantly down-regulated ACC2 more than did EPA. Correspondingly, the TG accumulations of the groups treated with these oxygenated fatty acids were significantly decreased more than did EPA or LA. The results from animal experiment also confirmed that these oxygenated fatty acids significantly reduced both forms of SREBP-1 protein in the liver of KK-Ay mice fed with HFD. In addition, oxygenated fatty acids containing ketone group also significantly decreased mRNA levels of SREBP-1c and ACC2 in the liver of those mice. These findings also supported that position and number of hydroxy group, ketone group, and double bond in fatty acids might play a crucial role in the effect of unsaturated or oxygenated fatty acids on the inhibition of cellular TG synthesis in liver cells, especially via SREBP-1c and ACC2 expression in this case.

Chapter 3: Oxygenated fatty acids fermented by *Lactobacillus plantarum* reduce intracellular lipid accumulation in 3T3-L1 cells

The oxygenated fatty acids, as mentioned in previous chapter, were treated to mouse embryonic fibroblast-adipose like cell line (3T3-L1) with differentiating medium. Cell viability was tested by WST-1 assay. The expression of CCAAT/enhancer binding protein β (C/EBP β), C/EBP α , peroxisome proliferator-activated receptor γ (PPAR γ) and SREBP-1c mRNAs were assessed by real-time RT-PCR. Phosphorylations of Akt were assayed by western blot analysis. TG synthesis in adipocytes was evaluated by Oil-red-O staining.

Those oxygenated fatty acids in this study significantly lowered lipid filling of differentiated 3T3-L1 cells, while EPA and LA did not. As expected, C/EBP β transcription was significantly down-regulated by most of oxygenated fatty acids used in this study at both 30 μ M and 60 μ M concentrations. PPAR γ transcription was decreased by all oxygenated fatty acids in this study. On the other hand, only oxo fatty acids can reduce the expression of C/EBP α . LA had no effects on the expression of PPAR γ and C/EBP α in this study. In addition, the phosphorylation of Akt after treatment with each fatty acid was examined. All fatty acids in this experiment did not differentiate the protein expression of pAkt compared with insulin-induced adipocytes. Taken together, this chapter proposes that these oxygenated fatty acids suppressed the adipogenesis via the down-regulation of C/EBP β and PPAR γ pathway, but not Akt phosphorylation. Interestingly, only oxo-fatty acids can down-regulate the expression of C/EBP α , while hydroxy-fatty acids, showed no significant effects.

SUMMARY AND CONCLUSION

It has been concerned that the stability of oxygenated fatty acids from autoxidation may be difficult to conserve. Moreover, the oxidized derivatives of EPA obtained from autoxidation contain numerous types and are hard to identify and purify for future use. In contrast, the fatty acids fermented by the enzymes of *L. plantarum* from fatty acids like linoleic acid, are more stable. In addition, these enzymes change the chemical structure at the specific location and function resulting in convenient identification and purification. Hence, it is interesting to oxygenate EPA with the enzyme of *L. plantarum* to get more stable and specific types of OEPA.

In conclusion, these data suggest that dietary some specific species of oxygenated fatty acids could be potentially useful as hypolipidemic and anti-obesity agents. The advantages of the oxygenated fatty acids in this study are also possible to connect with the prevention of many other metabolic and cardiovascular diseases. Furthermore, this study also discovered that number, location and type of hydroxy group, ketone group and double bond correlate with the efficiency of each fatty acid in lipid metabolism regulation. The author believes that this study would provide a significant opportunity to develop and utilize oxygenated fatty acids from marine sources for supplement or medicinal use. Nevertheless, the oxidation products always develop the worse smell than their substrates and it is difficult to relieve this drawback in the real food. However, their benefits are also very attractive. Therefore, to utilize them as supplement, the technology of capsule can prevent the bad smell to a consumer. In addition, using EPA extracted from algae or other sources, instead of fish, may reduce the fishy smell even though after oxidation.